Some Evidences of Premature Stoppage of Sugar Maple Sap Production

MAPLE SAP PRODUCTION during a season is often limited by the premature stoppage of sap flow. Quite often the dried tap holes show definite microbial infection. Recently, Naghski (1953) and Naghski and Willits (1955) concluded that this premature stoppage was attributable to the invasion of bacteria and possibly other microorganisms. Their conclusions, were based upon bacterial counts, no histological observations of the maple tissue being made. Since a true microbial invasion can usually be detected from histological slides and since an actual physical plugging should be reflected in histological materials if proper care is given, a thorough investigation of the tissue around normally tapped holes was carried out at selected intervals during the sap season of 1955. At this time there seemed to be no apparent change at the tissue level when the sap was free-flowing, yet signs of microbial invasion were clearly indicated from studies of the tissues around dried tap holes at the end of the sap season.

To further elucidate the mechanism of premature stoppage, an experiment was performed in 1956 to compare sap production and the degree of microbial infection following three kinds of tapping: (1) normal non-sterile tapping, (2) sterile tapping, and (3) sterile tapping followed by deliberate inoculation with known microorganisms.

This present paper reports the histological findings and some preliminary observations concerning the mechanism of stoppage of sugar maple sap production. Other results of this study, regarding bacterial counts, isolates, and sap production, will be published separately.

Materials and Methods

Histological study. Thirty sugar maple trees of 20-inch diameter or greater, located in the experimental woodlot of Michigan State University, were tapped in February, 1956. Each tree was triply tapped by the three types of tapping mentioned above. Sap production from each tap hole was recorded daily and bacterial counts of the sap were checked at definite intervals (this part of the work was done by the Departments of Forestry and Microbiology). Toward the middle of the sap season, 12 of the 30 experimental trees already showed a high degree of contamination within the inoculated

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tap holes, some being completely dried.

Materials for histological study were taken from trees of this contaminated group by keeping the two following criteria in mind: (1) only trees which produced approximately the same total amount of sap from all three tap holes normally expected from an equal sized tree were selected, and (2) only trees which showed a differential production of sap among the three specific tap holes were selected (sterile tap holes produced the highest total amount of sap, while inoculated ones yielded the least). Tissue around each tap hole was collected, half being fixed in FAA (15 parts formalin, 15 parts glacial acetic acid, 70 parts 70 percent ethanol, by volume), and the other half either sectioned as fresh material or kept frozen for later use. An additional group of samples having the same history was collected at the end of the sap season.

In order to study the actual condition of fresh materials from the three kinds of tapping, sections (approximately 40 μ thick) were cut with a freezing microtome modified for this purpose, stained with 0.1 percent neutral red and immediately examined for the relative infestation of microorganisms. Additional duplicate materials were killed and fixed, embedded in paraffin by standard histological procedures and cut into thinner sections (16-18 microns) with a Spencer sliding microtome. These sections were then stained either with Pianese IIIB or a modification of Conant's quadruple stain (Johansen, 1940), and prepared as permanent slides for detailed histological study.

Physiological study. Three bacterial cultures (*Pseudomonas* spp.) which had been isolated in 1955 from normally tapped holes, and used for the deliberate inoculation of trees in 1956 were inoculated into 50 ml of 0.3 percent beef-extract containing 0.1 percent pectin and incubated on a shaker for 72 hours at 25°C. Cells were then harvested from the liquid culture by centrifugation (10 minutes at 24,000 × g). After re-suspending the resulting bacterial pellets in 5 ml sterile glass-distilled water the suspension was tested for the formation of gummy substances and for the determination of pectolytic activity.

Sterilely collected and normally collected samples of sap were processed in the following manner: sterile sap was concentrated by alternate freezing and partial thawing to approximately 0.01 of its original volume, i.e., 2000 ml of sap was concentrated to a 20 ml volume, while normal sap was lyophilized and re-suspended in sterile water to a final concentration of 5×.

Results

Normal non-sterile tapping. From the fresh, frozen sections marked infestation by yeasts, molds, and bacteria was clearly evident. Thick crusts of microflora lined the dried tap holes. These crusts were composed of a network of mycelium (both septate and non-septate branching hyphae) with scattered yeast cells and gummy deposits containing tremendous numbers of bacteria (bacilli, 2-4 μ in length, 0.2-0.5 μ in diameter; cocci, 0.5 μ in diameter) (Fig. 1). Only remnants of the microflora, however, could be found in the permanent histological preparations. As a result of the numerous washings and dehydrations during the standard paraffin embedding and staining,

FIGURE 1. *Fresh longitudinal section of normally tapped maple tissue showing septate hypha (a), yeast cells (b), and bacteria embedded in dark gummy substances (c).*

only a small percentage of the original population was still present.

Within the tissues around the tap hole, yeast cells were sporadically found in vessel elements and living cells adjacent to the larger vessels. Their numbers became enormous toward the end of the season. Bacteria were "embedded" in gummy substances which partially or wholly filled the vessel lumens and chambers of bordered and half-bordered pits of the vessel walls (Fig. 2). Bacteria were also observed scattered through all types of cells and at times penetrating to a depth of 5 mm above or below the tap hole, but more limited in their horizontal spread. Fungal hyphae were occasionally seen in vessels and other types of cells (Fig. 3).

Sterile tapping. From the frozen sections no sign of microorganisms could be detected. Only the dried sap, oxidized cellular contents, and tapping debris lined the tap holes. The same situation was true in the permanent (paraffin embedded) slides.

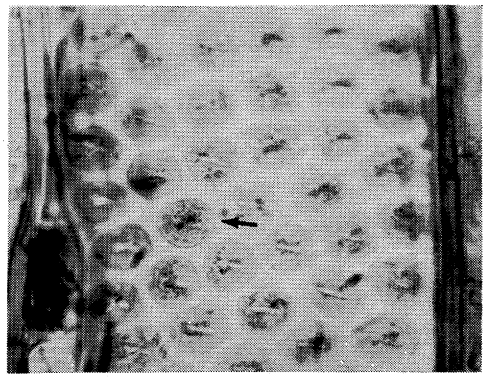

FIGURE 2. *Permanent longitudinal section of vessel wall showing bacteria-infested chambers and cannals of bordered pits.*

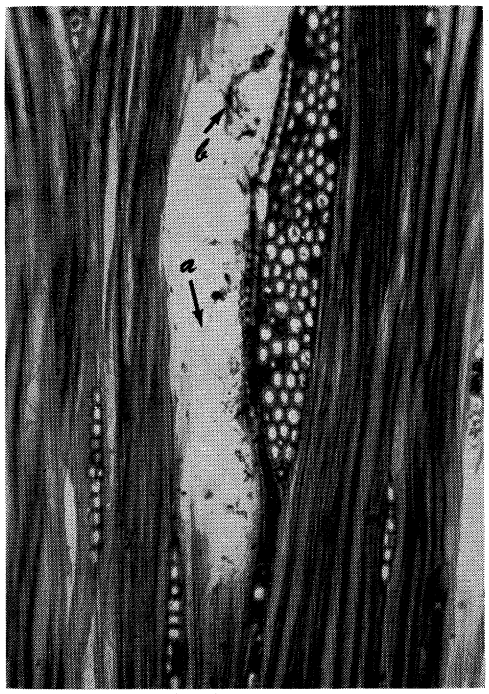

FIGURE 3. *Permanent tangentional section of tissue 2 mm. from tap-hole showing infested fungal hypha (a), and bacteria embedded in gummy substances (b) in vessel.*

Inoculated tapping. Bacterial invasion was well demonstrated within tissue surrounding the tap holes of this material. The organisms were congested in vessel elements and had invaded the neighboring parenchymatous tissues as well as the wood fibers (Fig 4). The torus and primary wall between adjoining bordered pits were dissolved and gummy substances were formed in pit channels and pit chambers (Fig. 5). Infested areas at times extended 6-7 mm below the tap holes, but to a lesser extent above them.

The Mechanism of Premature Stoppage

Vessel blockage. The open lumens of vessel elements surrounding the inoculated and normal non-sterile tap holes were prevailingly plugged with gummy substances, brownish in color and containing numerous bacteria. These substances are evidently produced by the invading organisms alone or from an interaction between the organisms and the maple tissue and sap. An attempt was made, therefore, to determine the relationship, if any, between the formation of the gummy residue and maple sap.

For this purpose, 2.5 ml of concentrated maple sap (collected by sterile tapping) was incubated at 30°C for 24 hours with 5 ml of the previously isolated bacterial suspension. Results in Table 1 show that vitality of the bacterial suspension is not essential but that non-boiled sap is necessary, for the formation of a gummy residue. This gummy residue was found to have the same staining properties and apparent physical consistency as the gummy plugs within vessel elements of the maple tissue. It thus seems probable that the gummy material composing the plug is induced by the bacteria and a normal constituent of the maple sap.

When concentrated normal (non-sterile) sap, containing yeast cells as well as bacteria, was tested in a similar manner for the formation of gummy substances, a larger quantity of residue was obtained.

TABLE 1. Results of incubation of bacterial suspensions and sterilely collected, concentrated maple sap.

Bacterial suspension	Maple sap		
	None	Boiled	Unboiled
None	.....	Precipitate	Albuminous
Boiled	Milky	Precipitate	Gummy residue
Unboiled	Milky	Precipitate	Gummy residue

Bacterial penetration into living cells. The observation that bacteria occurred on both sides of the bordered pits and half-bordered pits indicates a true invasion of plant tissue by the microorganisms. This has also been shown to be the case in verticillium wilt and bacterial wilt in plants, wherein the microorganisms dissolve cell walls by the action of pectinases (Kelman 1953, Scheffer *et al.* 1956). Therefore, an attempt was made

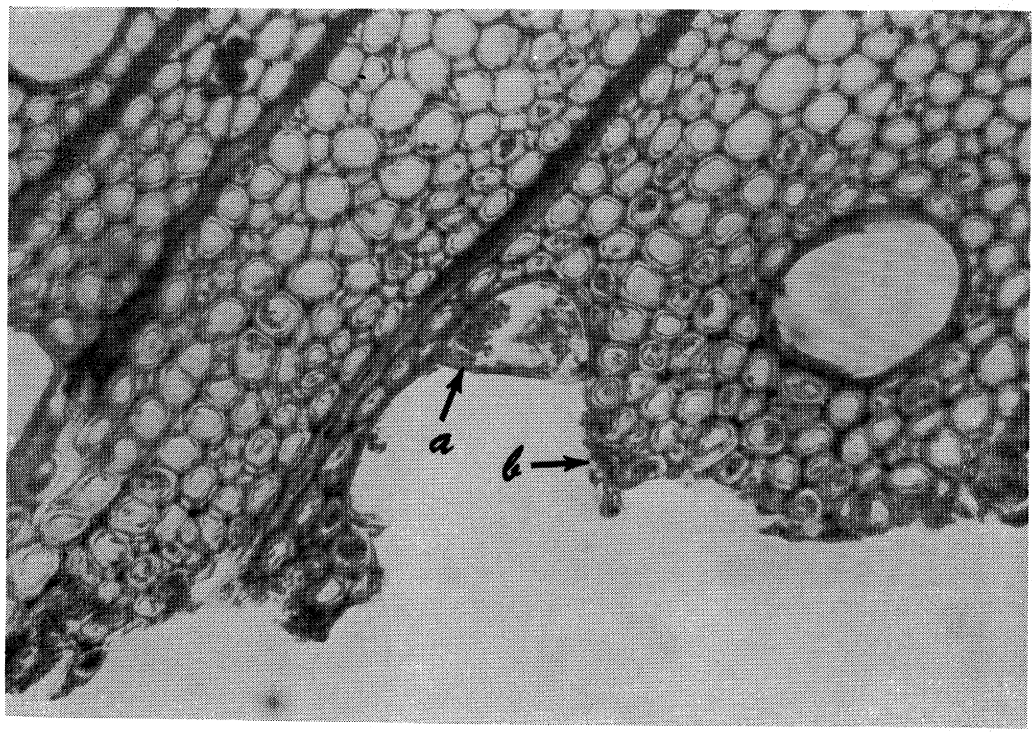

FIGURE 4. *Permanent cross section of tissue around innoculated tap-hole showing the abundance of yeast cells (a) and bacteria embedded in gummy substances (b) in vessels, fibers and ray cells.*

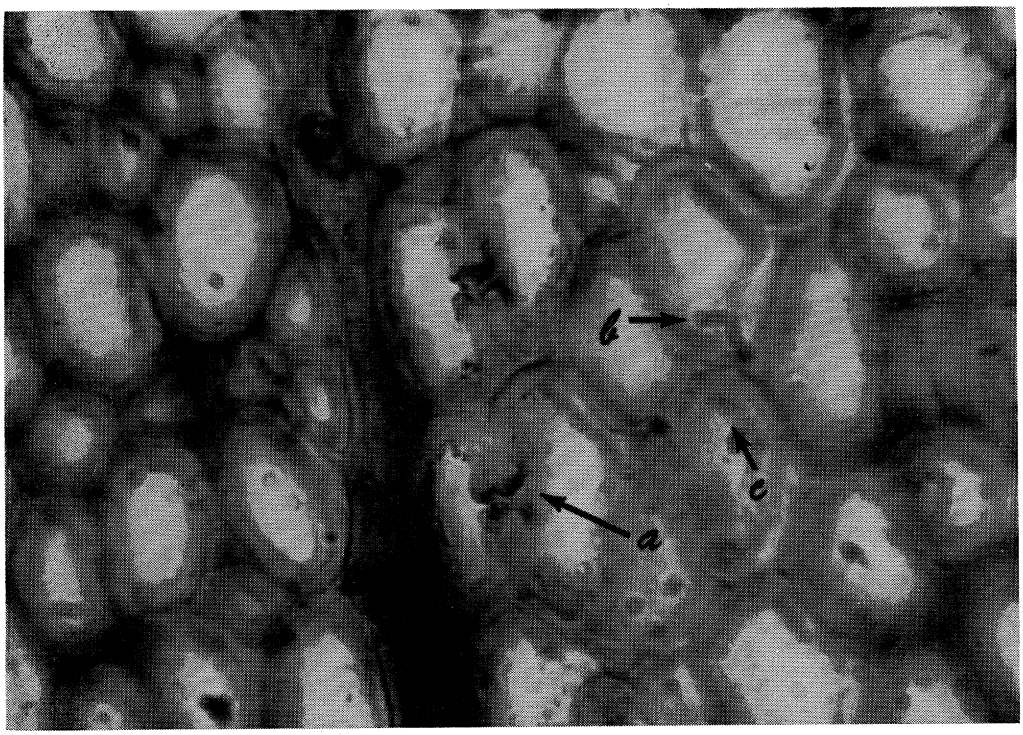

FIGURE 5. *Permanent cross section of infested tissue showing irregular cell lumens of fibers, dissolved cell wall with gummy plug (a) fungal hypha (b), bacteria (c), and gummy substances in ray cells.*

to demonstrate pectolytic activity of the isolated bacteria by utilizing a standard method (Bell *et al.* 1950) of measuring the viscosity changes in a pectin solution. An average of ten percent reduction in viscosity was observed in a non-boiled as compared with a boiled suspension after 45 minutes incubation at 30°C. Conceivably, even greater reduction might have been obtained by increasing the incubation time and increasing the pH as has been pointed out by Kelman (1953) and Kertesz (1951). It seems reasonable, therefore, that these microorganisms possess pectolytic activity and that this activity may provide a mechanism by means of which pathogenic penetration is accomplished.

Discussion and Conclusions

Observations that bacteria can induce the formation of gummy substances in vitro and that these apparently are the same gummy substances which make up the plugs within vessel elements which surround normal and inoculated tap holes indicates the possibility of an actual physical plugging of the sap ascending pathways. Similar gummy plugs containing bacteria have been observed in various species of plants infected by bacterial wilt, *Pseudomonas solanacearum*, whereby plugging hinders water transport in the plant, then wilting occurs (Kelman 1953). The same mechanism may hold true in the case of sugar maple.

In addition to the physical plugging, a physiological disturbance may also be indicated by the fact that bacteria were present in all types of cells adjacent to the vessel elements. These bacteria apparently enter the open lumens of cells surrounding the tap hole, thence invade the living cells via adjacent pits conceivably as a result of their pectinase activity. In spite of the different opinions regarding the mechanisms of sap

flow (Johnson 1945, Marvin and Erickson 1956, Marvin and Greene 1951, Stevens and Eggert 1945, Wiegand 1906), many workers in the field consider living cells to be the vital controlling centers. Since bacteria can readily invade living cells of the maple tissue, it is reasonable, therefore, to believe that the physiological functions of such cells might become so altered that normal sap flow would be affected.

Microorganisms other than bacteria are probably not directly responsible for *premature* stoppage. The appearance of yeast cells is limited to only the warmer weather toward the end of the sap season and their penetration into the tissues apparently follows bacterial invasion. The scarcity and inconsistent localization of fungal materials limits their significance in premature stoppage, although they may exert a secondary effect near the end and/or after the sap season, causing infection and discoloration of the tissues.

Summary

Histological findings indicate that premature stoppage of sap flow in normally tapped maple trees results from the combined effects of a bacterial invasion of the living tissue via pits along the cell walls, and an actual vessel blockage by microorganisms and/or gummy plugs produced by these microorganisms. Demonstration of pectolytic activity in the bacteria isolated from tap holes suggests a mechanism whereby these organisms penetrate the pit membranes.

Literature Cited

Bell, T. A., J. L. Etchells, and I. P. Jones. 1950. Softening of commercial cucumber salt-stock in relation to polygalacturonase activity. Food Tech. 4: 157-163.

Johansen, D. A. 1940. Plant Microtechnique. McGraw-Hill Book Co., New York.

Johnson, L. P. V. 1945. Physiological studies on sap flow in the sugar maple, *Acer saccharum* Marsh. Can. J. Res. 23: 192-197.

Kelman, A. 1953. The bacterial wilt caused by *Pseudomonas solanacearum*. N. Carolina Agric. Expt. Sta. Tech. Bull. No. 99.

Kertesz, Z. I. 1951. Pectic enzymes. In: The Enzymes, Ed. by J. B. Sumner and K. Myrback. Academic Press, New York.

Marvin, J. W., and R. O. Erickson. 1956. A statistical evaluation of some of the factors responsible for the flow from the sugar maple. Plant Physiol. 31: 57-69.

———, and M. T. Greene. 1951. Temperature-induced sap flow in excised stems of *Acer*. Plant Physiol. 26: 565-580.

Naghski, J. 1953. The organisms of maple sap: their effect and control. Proc. Second Conf. on Maple Products. Agric. Res. Ser., U. S. Dept. of Agric.

———, and C. O. Willits. 1955. Maple sirup. IX. Microorganisms as a cause of premature stoppage of sap flow from maple tap holes. Appl. Microbiol. 3: 149-151.

Scheffer, R. P., S. S. Gothoskar, and R. P. Collins. 1956. Physiological aspects of *Verticillium* wilt. Phytopath. 46: 83-87.

Stevens, C. L., and R. L. Eggert. 1945. Observations on the causes of flow of sap in red maple. Plant Physiol. 20: 636-648.

Wiegand, K. M. 1906. Pressure and flow of sap in maple. Amer. Nat. 40: 409-453.